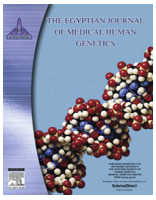
The Egyptian Journal of Medical Human Genetics 18 (2017) 389–392

HOSTED BY

Contents lists available at ScienceDirect

The Egyptian Journal of Medical Human Genetics

journal homepage: www.sciencedirect.com



Original article

Extremely low frequency electromagnetic field in combination with β-Lapachone up-regulates the genes of non-homologous end joining

Fatemeh Sanie-Jahromi, Hamideh Mahmoudinasab, Mostafa Saadat *

Department of Biology, College of Sciences, Shiraz University, Shiraz, Iran

ARTICLE INFO

Article history:
Received 9 May 2017
Accepted 30 May 2017
Available online 12 June 2017

Keywords:
β-Lapachone
Electromagnetic field
Morphine
NHEJ
Gene expression

ABSTRACT

Background and purpose: Non-homologous end joining (NHEJ) is the major pathway for removing DNA double strand breaks lesions. NHEJ is considered to be a resistant factor against chemotherapy induced neuropathy. β-Lapachone (β-Lap) is one of the antineoplastic agents which is shown to have anti neuroinflammatory effects. Extremely low frequency (<300 Hz) electromagnetic field (EMF) is shown to decrease NHEJ genes expression. Morphine (Mor) is associated with reducing effect on DNA repair and induce DNA damages. The goal of this study was to evaluate the effect of combination treatment of β-Lap, morphine (Mor) and EMF on expression of NHEJ related genes (*XRCC4*, *Ku70*, *Ku80*, *DNA-PKcs* and *LIG4*).

Materials and methods: SH-SY5Y cells (epithelial neuroblasts) were treated with four combinational treatments of β-Lap (2.0 and 3.2 μM), Mor (5.0 μM) and EMF (50 Hz, 0.50 mT, "15 min field on/15 min field off") and mRNA levels of *XRCC4*, *Ku70*, *Ku80*, *DNA-PKcs* and *LIG4* were evaluated by quantitative real-time PCR and primers specific for the examined genes. The experiments were done in triplicates.

Results: No significant alteration in the mRNA levels of NHEJ related genes was observed in "β-Lap alone" and "β-Lap + Mor" treated cells. The expression levels of NHEJ related genes were significantly increased in "β-Lap + EMF" and "β-Lap + Mor + EMF". Multiple linear regression analysis showed that the effect of EMF and Mor on NHEJ related genes expression is opposite to the effect of β-Lap.

Conclusion: In overall, combination of β-Lap, Mor and EMF leads to increased expression of NHEJ related gene expression. This effect may lead to decreased sensitivity of SH-SY5Y cells against β-Lap and can improve its neuroprotective property which might be hopeful for its clinical applications.

1. Introduction

β-Lapachone (β-Lap), is a natural naphthoquinone anticancer drug which is activated by NAD(P)H quinone oxidoreductase 1 (NQO1) and generates hydroquinone and ROS [1]. β-Lap has several special characteristics such as anti-neoplastic and anti-neuroinflamatory [2] properties to which much attention has focused. Different studies have shown that β-Lap exerts cytotoxic effect via its inhibitory effect on topoisomerase I and induction of ROS generation which ultimately leads to DNA double strand breaks [3,4]. Non-homologous end joining (NHEJ) is the major pathway for removing DNA double strand breaks lesions around the cell cycle and is known to be responsible for repair of nearly 80% of double strand breaks [5]. The most important components involved in NHEJ pathway include DNA-PKcs (OMIM: 600899), Ku70 (OMIM: 152690), Ku80 (OMIM: 194364), LIG4 (OMIM: 601837), and XRCC4 (OMIM: 194363). There are evidences which indicate that β-Lap efficiency can be affected by NHEJ performance [6].

Extremely low frequency (<300 Hz) electromagnetic field (EMF) was shown to decrease NHEJ genes expression [7]. Morphine (Mor) is also reported to have reducing effect on DNA repair [8], induce DNA damages [9], and down-regulate several antioxidant genes [10,11]. Different studies have shown that changes in DNA repair capacity is the cornerstone in defining cell response to DNA damaging drugs such as β-Lap [12]. On the other hand DNA repair can play pivotal role for protecting against chemotherapy induced neuropathies [13]. To our knowledge there is no study on the effect of combination treatment of β-Lap, Mor, and EMF on the transcript levels of NHEJ related genes. This study was conducted to investigate the expression levels of NHEJ related genes in human SH-SY5Y cells under treatments of "β-Lap alone", "β-Lap + Mor", "β-Lap + EMF" and "β-Lap + Mor + EMF". SH-SY5Y cell line corresponds to epithelial neuroblasts which are known as an experimental cell models for *in vitro* studies of neural functions.

Peer review under responsibility of Ain Shams University.

* Corresponding author.
E-mail addresses: fsanie@shirazu.ac.ir (F. Sanie-Jahromi), hnahmoudinasab@yahoo.com (H. Mahmoudinasab), saadat@shirazu.ac.ir, msaadat41@yahoo.com (M. Saadat).

http://dx.doi.org/10.1016/j.ejmhg.2017.05.003
1110-8630/

2. Materials and methods

2.1. Cell culture

SH-SY5Y neuroblastoma cell line was obtained from Pasteur Institute of Iran and cultured in DMEM/F12 enriched with 10% FBS (Gibco), penicillin (100 U/ml, Sigma) and streptomycin (100 μg/ml, Sigma). Cultures were maintained in a 37 °C incubator with a 5% CO_2, 95% air atmosphere.

2.2. EMF exposure

The exposure apparatus was consisted of a solenoid working with 50-Hz sinusoidal alternating current to generate 0.50 mT EMF in the intermittent pattern of "15 min field on/15 min field off" [14]. The total time for EMF exposure was 30 min. No temperature change was observed during EMF exposure.

2.3. Treatments

Four treatments were applied in this study as followed: 1) β-Lap alone; 2) "β-Lap + Mor"; 3) "β-Lap + EMF"; 4) "β-Lap + Mor + EMF". Two concentrations of β-Lap (2.0 and 3.2 μM, Sigma-Aldrich) and a single dose of Mor (5.0 μM, LGS standards, Swiss) were used in this experiment. SH-SY5Y cells were seeded at 3×10^5 cells/ml and incubated at 37 °C for 24 h. For "β-Lap + EMF" treatment, the cells were treated with β-Lap and subsequently exposed to EMF. For "β-Lap + Mor + EMF" treatment, cells were treated with β-Lap and Mor and then exposed to EMF. Cells which were treated with "β-Lap alone" or "β-Lap + Mor", were located in the EMF apparatus, for the same time when the power was off. In all treatments, cells were harvested after 24 h and then RNA extraction was done.

2.4. RNA extraction, cDNA synthesis and real time PCR

RNA extraction, cDNA synthesis, quantitative real-time PCR and primers specific for the examined genes were described before [7]. Quantitative real time PCR analysis was carried out using syber® premix Ex Taq™ II (Takara Bio Inc., Japan) in Rotor-Gene 6000 instrument (Corbett research). Relative gene expression was analyzed using $2^{-\Delta\Delta Ct}$ method [15].

2.5. Statistical analysis

The experiments were done in triplicates. Data were shown as means ± standard error (SE). Alterations in mRNA levels were

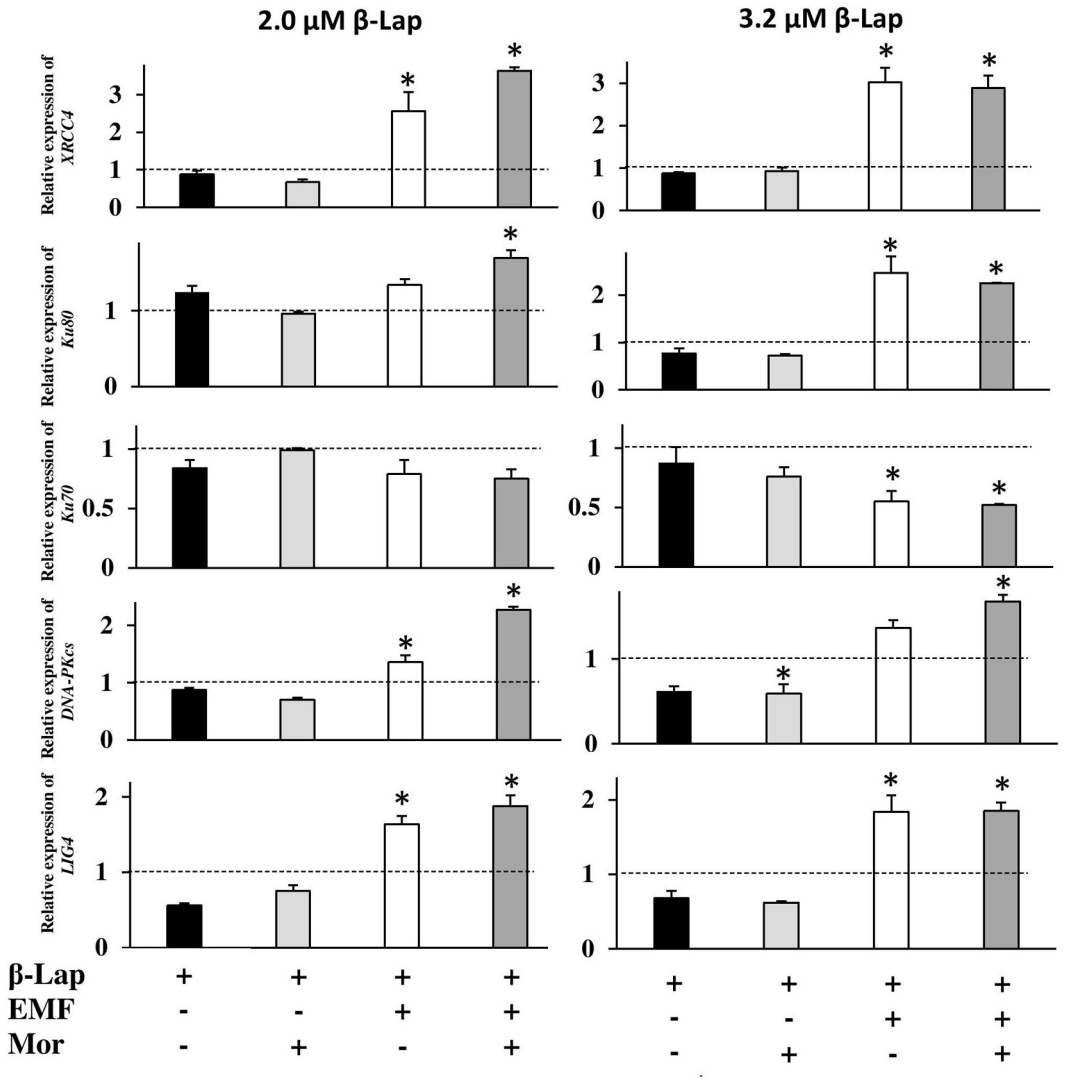


Fig. 1. mRNA levels of the examined genes of non-homologous end joining pathway in "β-Lap" (β-Lapachone, at 2.0 and 3.2 μM), "β-Lap + Mor", "β-Lap + EMF" and "β-Lap + Mor + EMF" treated cells. (EMF: electromagnetic field, Mor: morphine, 5.0 μM). n = 3, mean ± SE. *P < 0.05 all values compared with control cultures using Bonferroni post hoc test.

Table 1
Results of multiple linear regression analysis of non-homologous end joining related transcript levels in human SH-SY5Y cells when the cells were exposed to combination treatment of β-Lap (β-Lapachone, at 2.0 and 3.2 μM), EMF (electromagnetic fields), and Mor (morphine, 5.0 μM). The treatments are mentioned in the text.

	XRCC4		
	β*	t	P
β-Lap	−0.056	−0.70	0.491
Mor	0.076	0.97	0.339
EMF	0.937	12.0	<0.001
	Adjusted R^2 = 0.854; F = 51.6; df = 3, 23; P < 0.001		
	Ku80		
	β*	t	P
β-Lap	0.099	0.74	0.462
Mor	−0.068	−0.52	0.605
EMF	0.778	6.04	<0.001
	Adjusted R^2 = 0.599; F = 13.9; df = 3, 23; P < 0.001		
	Ku70		
	β*	t	P
β-Lap	−0.423	−2.77	0.011
Mor	0.029	0.19	0.844
EMF	−0.500	−3.38	0.003
	Adjusted R^2 = 0.470; F = 8.6; df = 3, 23; P < 0.001		
	DNA-PKcs		
	β*	t	P
β-Lap	−0.327	−3.53	0.002
Mor	0.238	2.65	0.014
EMF	0.903	10.0	<0.001
	Adjusted R^2 = 0.805; F = 36.6; df = 3, 23; P < 0.001		
	LIG4		
	β*	t	P
β-Lap	−0.146	−1.99	0.058
Mor	0.055	0.76	0.450
EMF	0.967	13.6	<0.001
	Adjusted R^2 = 0.878; F = 64; df = 3, 23; P < 0.001		

* Standardized coefficient.

analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni post hoc test. A multiple linear regression analysis was conducted to determine the effect of β-Lap, Mor and EMF on the expression levels of NHEJ related genes. Statistical analysis was done using SPSS statistical software package (v. 20, SPSS Inc. Chicago, IL, USA). A probability of P < 0.05 was considered statistically significant.

3. Result

The relative IC_{50} value of β-Lap was estimated 3.54 (95% CI: 3.45–3.63) μM. In the present study we used β-Lap at two final concentrations: 2.0 and 3.2 μM. It should be noted that 3.2 μM β-Lap showed about 18 percent cytotoxicity, whereas 2.0 μM β-Lap showed no cytotoxicity.

Fig. 1 shows the alteration of mRNA levels of NHEJ related genes in "β-Lap alone", "β-Lap + Mor", "β-Lap + EMF" and "β-Lap + Mor + EMF" treatments. No significant alteration in the mRNA levels of NHEJ related genes was observed in "β-Lap alone" and "β-Lap + Mor" treated cells. The mRNA levels of NHEJ related genes were significantly increased in "β-Lap + EMF" and "β-Lap + Mor + EMF". *XRCC4*, *Ku80*, *DNA-PKcs* and *LIG4* were the most up-regulated genes in 2.0 and 3.2 μM β-Lap combined with EMF or "Mor + EMF". *Ku70* was the only gene whose expression was not increased and in fact it was decreased when EMF was applied in combination with 3.2 μM β-Lap (Fig. 1).

Multiple linear regression analysis showed that EMF was positively associated with the expression levels of *XRCC4*, *Ku80*, *DNA-PKcs* and *LIG4* and negatively associated with the *Ku70* expression level. Mor had positive effect on *DNA-PKcs* transcript level. It should be noted that concentration of β-Lap had negative effect on the mRNA levels of *Ku70* and *DNA-PKcs* (Table 1).

4. Discussion

At present time, Mor is widely administered for pain relief in cancer patients receiving chemotherapy [16,17]. Studies have shown that Mor might be associated with oxidative stress and DNA damage [9,18–20]. Extremely low frequency electromagnetic field (EMF) is a non-ionizing and non-thermal power which can penetrate the cell and affect many cellular functions including alterations in expressional levels target genes [21]. We showed that the 50 Hz EMF (0.50 mT, "15 min field on/15 min field off") can modulate the mRNA levels of genes related to DNA repair pathways and subsequently alter cellular toxicity to cisplatin, an alkylating agent [7]. Today chemotherapy drugs and Mor are simultaneously used [22] and peripheral neuropathy is one of the important side effects of chemotherapy regiments [23]. Considering that alterations in NHEJ is associated with the efficiency of treatment [12,24–26] and there is no published study on the effect of combination treatment of β-Lap, Mor and EMF on transcript level of NHEJ related genes, the present experiments were carried out.

Although treatment with each of extremely-low frequency of EMF [6] and Mor [7] has been reported to reduce transcript level of NHEJ genes, our present results showed that EMF was associated with increasing the mRNA levels of NHEJ related genes (Table 1). It might be concluded that the effect of EMF (and also Mor) on the expression of NHEJ related genes is opposite to the effect of β-Lap. Hence combination of β-Lap, Mor and EMF leads to increased expression levels of the examined NHEJ related genes. This elevation may lead to increased repair of double strand breaks. Recently it has been shown that inhibition of NHEJ could strengthen β-Lap lethality [5]. There are some reports which indicate that β-Lap has a preventive neuroinflammatory property which is hopeful for eliminating neural degeneration during treatments [2]. Since SH-SY5Y is a suitable model for studying chemotherapy-induced neuropathies, our data suggest that the empowered capacity of DNA repair of double strand breaks may lead to decreased sensitivity of SH-SY5Y cells and improve the neuroprotective properties of β-Lap in combination with EMF.

5. Conclusion

In this study SH-SY5Y cells were applied as an *in vitro* model for studying chemotherapy-induced neuropathies. According to the presented results, combination treatment of β-Lapachone, morphine and extremely low frequency (50-Hz) electromagnetic field leads to increased expression of non-homologous end joining (NHEJ) related genes. Considering the important role of DNA repair capacity for protecting against neural degeneration, it is suggested that extremely low frequency electromagnetic field treatment can improve the neuroprotective properties of β-Lapachone in SH-SY5Y cells. Further *in vivo* experiments should be conducted to confirm our present results.

Disclosure statement

The authors declare no conflict of interest.

Acknowledgement

This study was supported by Shiraz University, Iran.

References

[1] Reinicke KE, Bey EA, Bentle MS, Pink JJ, Ingalls ST, Hoppel CL, et al. Development of beta-lapachone prodrugs for therapy against human cancer cells with elevated NAD(P)H:quinone oxidoreductase 1 levels. Clin Cancer Res 2005;11:3055–64.

[2] Lee EJ, Ko HM, Jeong YH, Park EM, Kim HS. B-Lapachone suppresses neuroinflammation by modulating the expression of cytokines and matrix metalloproteinases in activated microglia. J Neuroinflamm 2015;12:133.

[3] Boothman DA, Pardee AB. Inhibition of radiation-induced neoplastic transformation by beta-lapachone. Proc Natl Acad Sci USA 1989;86:4963–7.

[4] Hori T, Kondo T, Lee H, Song CW, Park HJ. Hyperthermia enhances the effect of β-lapachone to cause γH2AX formations and cell death in human osteosarcoma cells. Int J Hyperther 2011;27:53–62.

[5] DiBiase SJ, Zeng ZC, Chen R, Hyslop T, Curran Jr WJ, Iliakis G. DNA-dependent protein kinase stimulates an independently active, non-homologous, end-joining apparatus. Cancer Res 2000;60:1245–53.

[6] Bentle MS, Reinicke KE, Dong Y, Bey EA, Boothman DA. Nonhomologous end joining is essential for cellular resistance to the novel antitumor agent, beta-lapachone. Cancer Res 2007;67:6936–45.

[7] Sanie-Jahromi F, Saadat I, Saadat M. Effects of extremely low frequency electromagnetic field and cisplatin on mRNA levels of some DNA repair genes. Life Sci 2016;166:41–5.

[8] Couch DB, Sawant SG. The clastogenicity of morphine sulfate in vivo. Adv Exp Med Biol 1995;373:123–9.

[9] Tsujikawa H, Shoda T, Mizota T, Fukuda K. Morphine induces DNA damage and P53 activation in CD3+ T cells. Biochim Biophys Acta 2009;1790:793–9.

[10] Saify K, Saadat I, Saadat M. Down-regulation of antioxidant genes in human SH-SY5Y cells after treatment with morphine. Life Sci 2016;144:26–9.

[11] Saify K, Saadat M. Expression levels of antioxidant genes in human SH-SY5Y cells long term exposed to methadone. Turk J Biochem 2016;41:493–4.

[12] Helleday T, Petermann E, Lundin C, Hodgson B, Sharma RA. DNA repair pathways as targets for cancer therapy. Nat Rev Cancer 2008;8:193–204.

[13] Dzagnidze A, Katsarava Z, Makhalova J, Liedert B, Yoon MS, Kaube H, et al. Repair capacity for platinum-DNA adducts determines the severity of cisplatin-induced peripheral neuropathy. J Neurosci 2007;27:9451–7.

[14] Mahmoudinasab H, Sanie-Jahromi F, Saadat M. Effects of extremely low-frequency electromagnetic field on expression levels of some antioxidant genes in human MCF-7 cells. Mol Biol Res Commun 2016;5:77–85.

[15] Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C_T method. Nat Protoc 2008;3:1101–8.

[16] Vallejo R, Barkin RL, Wang VC. Pharmacology of opioids in the treatment of chronic pain syndromes. Pain Physician 2011;14:E343–60.

[17] Lever JR. Opioid receptors and ligands: targets for cancer imaging and therapy. Med Chem 2012;2:142–6.

[18] Aminian A, Javadi S, Rahimian R, Dehpour AR, Asadi Amoli F, Moghaddas P, et al. Enhancement of cisplatin nephrotoxicity by morphine and its attenuation by the opioid antagonist naltrexone. Acta Med Iran 2016;54:422–9.

[19] Shafer DA, Xie Y, Falek A. Detection of opiate-enhanced increases in DNA damage, HPRT mutants, and the mutation frequency in human HUT-78 cells. Environ Mol Mutagen 1994;23:37–44.

[20] Zhang YT, Zheng QS, Pan J, Zheng RL. Oxidative damage of biomolecules in mouse liver induced by morphine and protected by antioxidants. Basic Clin Pharmacol Toxicol 2004;95:53–8.

[21] Funk RHW, Monsees T, Özkucur N. Electromagnetic effects-from cell biology to medicine. Prog Histochem Cytochem 2009;43:177–264.

[22] Hanks GW, Conno F, Cherny N, Hanna M, Kalso E, McQuay HJ, et al. Morphine and alternative opioids in cancer pain: the EAPC recommendations. Br J Cancer 2001;84:587–93.

[23] Donzelli E, Carfì M, Miloso M, Strada A, Galbiati S, Bayssas M, et al. Neurotoxicity of platinum compounds: comparison of the effects of cisplatin and oxaliplatin on the human neuroblastoma cell line SH-SY5Y. J Neurooncol 2004;67:65–73.

[24] Madhusudan S, Hickson ID. DNA repair inhibition: a selective tumor targeting strategy. Trends Mol Med 2005;11:503–11.

[25] Begg AC, Stewart FA, Vens C. Strategies to improve radiotherapy with targeted drugs. Nat Rev Cancer 2011;11:239–53.

[26] Salles B, Calsou P, Frit P, Muller C. The DNA repair complex DNA-PK, a pharmacological target in cancer chemotherapy and radiotherapy. Pathol Biol (Paris) 2006;54:185–93.